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RESEARCH HIGHLIGHT

How to correct chromosomal trisomy

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Trisomy 21 in human causes Down syndrome, a common chromosome disorder with devastating phenotypes ranging from early death *in utero* to intellectual disability together with an array of physical anomalies and late-onset diseases. In a recent study published in *Nature*, Jeanne Lawrence and her colleagues restored normal gene expression in trisomy 21 cells by silencing the extra chromosome using *XIST*, the non-coding RNA that normally silences one X chromosome in females; this improved growth and differentiation of neural cells, which offers hope that some deleterious effects of the trisomy could be reversed to improve this incurable disease.

Global gene expression analyses show that not only chromosome 21-linked genes but also genes on other chromosomes are disrupted in cells with trisomy 21, suggesting wide effects on the transcriptome. Because such comparisons are usually between different individuals and not between isogenic cells, a large portion of the changes observed could be due to variants unrelated to the trisomy. In fact, few specific genes have been implicated in the trisomy phenotypes, and the effects of abnormal gene dosage are not clear in different tissues. The fold-increase in expression for genes on chromosome 21 is about 1.2-1.4 and only reaches the theoretical 1.5 for a minor portion of genes in trisomic cells, suggesting that dampening effects partially correct abnormal expression levels. Basal dampening and feedback mechanisms mitigate deleterious dosage effects of aneuploidy [1, 2]. To achieve correct gene expression, additional feed-forward mechanisms of dosage compensation evolved, for example to regulate the X chromosome. Such mechanisms include X upregulation to increase expression from the single active X chromosome, and X inactivation to silence one X chromosome in mammalian females [1].

Lawrence and colleagues reasoned that dosage compensation by X inactivation could be appropriated to correct trisomy 21 [3] (Figure 1). X inactivation is initiated by *XIST*, a long noncoding RNA that encases the inactive X chromosome in a silent compartment. Harnessing *XIST* power to silence one chromosome 21 and restore normal gene expression in trisomic cells was possible because *XIST* RNA spreads *in cis* even when inserted or attached to an autosome [4-6]. After insertion and induction of a large genomic fragment containing *XIST* in trisomic iPS cells using a ZFN system to target chromosome 21, Jiang *et al*. [3] observed the accumulation of repressive histone modifications together with DNA methylation of CpG islands. While these epigenetic changes appear stable in differentiated cells, maintenance of silencing of individual chromosome 21-linked genes after many cell divisions remains to be demonstrated. In transgenic mice with an *Xist* insert on an autosome, reactivation is often observed over time, and silencing is patchy in autosomal portions of human X;autosome translocations. In contrast, X inactivation is very stable, suggesting that X-specific elements absent on autosomes may have evolved to help stabilize silencing. Interestingly, cells corrected by *XIST* insertion on chromosome 21 grow better than control trisomic cells and are more efficient in the formation of neural rosettes [3]. Since the *XIST* insert is within the *DYRK1A* gene whose dosage is critical for neural differentiation [7], the observed improvement may be due to *DYRK1A* knockout rather than *XIST*-mediated epigenetic silencing. Cells with *XIST* inserts on two or even three of the chromosomes 21 rapidly lost *XIST* expression [3], consistent with monosomy or nullisomy 21 being incompatible with survival.

Could *XIST* insertion into any autosome be widely applied to trisomy rescue? In principle, such rescue could be achieved at the cellular level. The difficulty will be to apply the strategy to an *in vivo* situation. One can imagine that some rescue could be obtained in a specific tissue such as bone marrow. Allogenic bone marrow transplant after correction of the trisomy has been proposed as a mean to alleviate the propensity of developing leukemia in Down syndrome [8]. In this study, insertion of a selectable marker (TK-NEO) into the *APP* gene on chromosome 21 was used to induce loss of this chromosome [8] (Figure 1). Clearly, as a mean to rescue trisomic cells, complete loss of chromosome 21 would be preferable to silencing by *XIST* because dosage compensation may not be complete or stable over a long time. However, the *XIST* silencing approach appears efficient, and silencing is inducible, making the system versatile [3]. In mouse, hematopoeitic precursor cells are permissive to X inactivation upon *Xist* induction [9], but induction

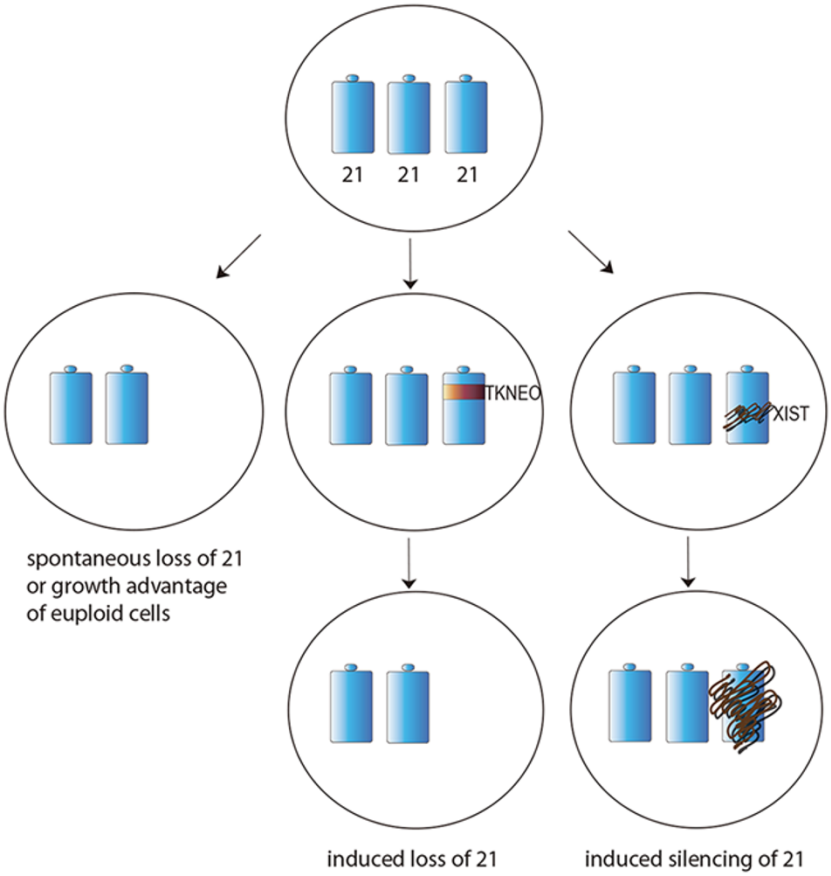

Figure 1 Trisomy 21 rescue. Euploid cells can arise from a population of trisomy 21 cells by several mechanisms: spontaneous loss of chromosome 21 together with growth advantage of euploid cells [12] (left); induced loss of chromosome 21 using a selectable marker (TKNEO) targeted to the *APP* gene [8] (middle); or induced silencing of one chromosome 21 by insertion of *XIST* into the *DYRKY1A* gene [3] (right).

in other cell types may be difficult, and fibroblasts were not as efficient as iPS cells in correcting trisomy 21 by *XIST* insertion [3]. Developmental defects associated with aneuploidy would be much more complicated to address due to difficulties in delivering a vector that carries *XIST*. Nonetheless, the approach may open ways to control the vast aneuploidy load observed in cancer cells. Since *XIST* spreads to entire chromosomes, barrier systems would have to be developed if only parts of a rearranged chromosome were to be silenced. On the X chromosome some genes escape silencing [10]. Such genes are often grouped within domains protected from epigenetic changes associated with silencing including *Xist* coating [4]. A better understanding of the insulator elements that separate domains would be helpful in designing ways to control *XIST* spreading.

One of the very useful output of the elegant study by Jiang *et al*. is to provide means to better understand Down syndrome in terms of the disruption of gene expression and of cellular phenotypes [3]. This very realistic prospect stems from the construction of pairs of isogenic cell lines, one with trisomy 21 and the other with a silenced chromosome 21, which can be rigorously compared to each other. In addition, stem cells (iPS) were derived, which can be differentiated in an array of cell types to address tissue-specific effects of the trisomy. So far, two cellular phenotypes were compared but others will be amenable to study. This may facilitate screens for drugs that relieve or worsen specific phenotypes to better treat Down syndrome individuals using a "personalized medicine" approach. Several mouse models of trisomy 21 have been derived, including one model with insertion of a copy of human chromosome 21 into a mouse. Such models have proven useful for rigorous comparisons of gene expression and phenotypes between disomic and trisomic mice with the same genetic background, and have helped develop drugs that alleviate some of the behavioral phenotypes [11]. The new resources developed promise to extend such studies to a human system [3].

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